# Evaluation of Distillation and Diffusion Techniques for Estimating Hydrolyzable Amino Sugar-Nitrogen as a Means of Predicting Nitrogen Mineralization

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A rapid method to estimate soil N mineralization to improve N fertilizer recommendations has long been sought. Over the years, numerous methods to predict N mineralization have been proposed, but no one method has been widely accepted. Recently, researchers observed the concentration of hydrolyzable amino sugar-N in the soil correlated with crop N response. The objective of this study was to determine if developmental methods that quantify hydrolyzable amino sugar-N accurately predict N mineralization when compared to net-N mineralization by anaerobic incubation. Methods evaluated to predict hydrolyzable amino sugar-N were the Illinois soil nitrogen test (ISNT) by diffusion, and 2, 5, and 10 $M$ NaOH direct steam distillation procedures. It was observed that the hydrolyzable amino sugar-N was a somewhat accurate predictor of N mineralization ($R^2$ = 0.38). However, when the hydrolyzable amino sugar-N concentrations were combined with the hydrolyzable $NH_4$–N concentrations, the ability to predict N mineralization improved ($R^2$ = 0.61). Suggesting more labile soil organic N forms along with amino sugar-N are potentially mineralizable. Rapid analytical procedures like the ISNT diffusion method and the 2, 5, and 10 $M$ NaOH direct steam distillation techniques accurately predicted hydrolyzable amino sugar-N as well as hydrolyzable ($NH_4$ + amino sugar)-N. These methods also accurately predicted $NH_4$–N mineralized after anaerobic incubation. It could be assumed that this predictability may increase when soils are analyzed based on soil management, geographic area, and crop rotation. Glucosamine recovery was significant ( >85%, $P$ = 0.05) for both methods showing their ability to quantify amino sugar-N in the soil as well as estimate the amount of potentially mineralizable-N. Lastly, in soil-testing facilities where the ISNT is already implemented as a procedure, the much quicker and equally reliable 10 $M$ NaOH distillation technique may be used to achieve near identical test values.

Abbreviations: ISNT, Illinois soil nitrogen test.

A routine soil test that accurately predicts the N supplying capability of a soil has long been sought. Over the years numerous biological and chemical methods have been proposed, but no one method has been widely accepted (Stanford, 1982). Researchers have attempted to examine specific organic N fractions that may become plant available throughout the growing season. Amino compounds are reportedly sensitive to organic amendments, such as manure, and may affect the native N fertility of a soil (Wander, 2004). Additionally, crop rotation may also affect concentrations of amino compounds in the soil. Praveen-Kumar et al. (2002) observed fields planted to legumes had increased amino acid and amino sugar concentrations, whereas, when pearl millet [*Pennisetum americanum* (L.) Leeke] was planted amino sugar concentrations decreased.

Mulvaney et al. (2001) evaluated specific fractions of hydrolyzable soil N as a means of predicting N mineralization. They determined conventional steam distillation methods for quantifying different fractions of hydrolyzable N, described by Stevenson (1996), were subject to serious error (Mulvaney and Khan, 2001). By using diffusion methods developed by Khan et al. (1997), total-N analysis of Kjeldahl digests (Stevens et al., 2000), and conducting recovery test of $^{15}N$ labeled compounds added to soil hydrolysates, they were able to accurately quantify specific fractions of hydrolyzable-soil N. They compared the specific fractions with corn (*Zea mays,* L.) response to N fertilization and observed significant correlations between the hydolyzable amino sugar-N concentration and both the check-plot yield and N fertilizer response.

Because the hydrolysis process and diffusion process were very time consuming and not practical for soil testing use, Khan et al. (2001) developed a procedure that accurately estimated the soil hydrolyzable amino sugar-N content. Their method consists of placing 1 g soil with 10 mL of 2 $M$ NaOH in a modified Mason jar for 5 h at 50°C. The time efficient diffusion method named the ISNT was significantly related with the hydrolyzable amino sugar-N, and significantly predicted fertilizer responsive from nonresponsive sites. Barker et al. (2006) observed that the ISNT was not well correlated with corn grain yield and corn grain yield response to N fertilization. It was also observed that the ISNT did not significantly

correlate with hydrolyzable amino sugar-N, but there was a significant correlation between it and hydrolyzable $NH_4$–N and hydrolyzable (amino sugar + $NH_4$)-N. Barker et al. (2006) concluded that the ISNT was thus estimating a more labile form of organic N along with amino sugar-N. Sharifi et al. (2007) evaluated the ISNT along with a direct steam distillation using 20 mL of 50% NaOH as means of predicting N mineralization. They observed significant correlations between the ISNT and a modified direct steam distillation procedure and N mineralized after a 24 wk aerobic incubation. A highly significant correlation between the ISNT and the modified direct steam distillation method was observed.

We hypothesized that hydrolyzable amino sugar-N would accurately predict N mineralization during an anaerobic incubation and according to Mulvaney et al. (2001), the ISNT would be significantly correlated with the hydrolyzable amino sugar-N method and thus would accurately predict N mineralization. Therefore, the objective of this study was to evaluate amino sugar-N as a means of predicting N mineralization by comparing it with the $NH_4$–N mineralized after a 14 d anaerobic incubation and to also evaluate diffusion and distillation procedures that estimate hydrolyzable amino sugar-N as a means of predicting N mineralization.

## MATERIALS AND METHODS
### Soil Samples

Twenty five surface soil samples (0–15 cm) with different characteristics were collected during the fall and winter months of 2003 and 2004 from agricultural sites across the south-central and mid-western United States. These agricultural sites included cultivated and pasture ecosystems with various management practices. Samples were initially oven-dried, crushed to pass a 2-mm sieve, and stored in air-tight containers. Texture for the soil samples was determined by the soil description in the appropriate county soil survey provided by the Natural Resources Conservation Service (NRCS). Total N was determined for each soil with the permanganate-reduced iron modification of the semi-micro Kjeldahl procedure (Bremner, 1996) and organic C using wet combustion techniques as described by Nelson and Sommers (1996). Inorganic $NH_4$–N and $NO_3$–N were determined by steam distillation techniques according to Mulvaney (1996). Soil water pH was measured with a glass electrode in a 1:2 soil weight to water volume mixture. The site and chemical characteristics for the 25 soil samples used in this study are presented in Tables 1 and 2, respectively.

### Anaerobic Incubation Procedures

To begin the anaerobic incubation process, 10 g of oven-dried soil was placed into a serum bottle along with 50 mL of double-deionized water. The bottle was sealed using a gastight septum stopper and $O_2$ was displaced from the bottle using $N_2$ gas. To ensure anaerobic conditions were achieved, five randomly selected soil samples were equipped with a combination redox electrode and the Eh was monitored throughout the incubation experiment. According to Patrick et al. (1996) aerated or oxidized soils typically have Eh values of +400 to +700 mV and anaerobic or reduced soils typically have Eh values from +400 to as low as –250 or –300 mV. All five randomly selected samples

Table 1. Site characterization of the 25 soils used.

| Soil no. | Series | Taxonomy Great Group | Texture† | Previous crop‡ |
|---|---|---|---|---|
| 1 | Pond Creek | Argiustolls | fsl | peanut |
| 2 | Pond Creek | Argiustolls | fsl | sorghum |
| 3 | Barco | Hapludults | l | corn |
| 4 | Osage | Epiaquerts | sic | bermudagrass |
| 5 | Osage | Epiaquerts | sic | soybean |
| 6 | Clarion | Hapludolls | l | corn |
| 7 | Webster | Endoaqualls | sicl | corn |
| 8 | Dewitt | Albaqualfs | sil | rice |
| 9 | Dewitt | Albaqualfs | sil | soybean |
| 10 | Tichnor | Endoaqualfs | sil | soybean |
| 11 | Tichnor | Endoaqualfs | sil | rice |
| 12 | Commerce | Endoaquepts | sil | soybean |
| 13 | Henry | Fragiaqualfs | sil | soybean |
| 14 | Dewitt | Albaqualfs | sil | soybean |
| 15 | Egam | Hapludolls | sicl | soybean |
| 16 | Sharkey | Epiaquerts | sic | rice |
| 17 | Pembroke | Paleudalfs | sil | fescue |
| 18 | Gruver | Paleustolls | cl | sorghum |
| 19 | Portland | Epiaquepts | c | cotton |
| 20 | Perry | Epiaquerts | c | rice |
| 21 | Portland | Epiaquepts | c | cotton |
| 22 | Portland | Epiaquepts | c | cotton |
| 23 | Sharkey | Paleudalfs | sic | soybean |
| 24 | Sharkey | Paleudalfs | sic | soybean |
| 25 | Sharkey | Paleudalfs | sic | rice |

† Determined from Natural Resources Conservation Service (NRCS) county soil survey. The suffix included with each series name indicates the textural class of the surface soil: fsl, fine sandy loam; l, loam; sic, silty clay; sicl, silty clay loam; sil, silt loam; cl, clay loam; c, clay.

‡ Peanut, (*Arachis hypogea* L.); sorghum, [*Sorghum bicolor* (L.) Moench]; corn, (*Zea mays* L.); bermudagrass, [*Cynodon dactylon* (L.) Per.]; soybean, [*Glycine max* (L.) Merr.]; rice, (*Oryza sativa* L.); fescue, (*Festuca arundinacea* Schreb.); cotton, (*Gossypium hirsutum* L.).

Table 2. Chemical characterization of the 25 soils used.†

| Soil no. | pH‡ | Organic C§ | Total N¶ | $NH_4$–N# | $NO_3$–N# |
|---|---|---|---|---|---|
| | | ——g kg$^{-1}$—— | | ——mg kg$^{-1}$—— | |
| 1 | 7.0 | 7.8 | 0.4 | 1.04 | 0.76 |
| 2 | 6.5 | 5.5 | 0.4 | 1.07 | 0.25 |
| 3 | 7.3 | 14.4 | 1.2 | 1.74 | 1.33 |
| 4 | 6.6 | 22.0 | 2.1 | 1.82 | 0.30 |
| 5 | 6.6 | 19.4 | 1.8 | 0.78 | 0.86 |
| 6 | 6.8 | 17.8 | 1.6 | 1.22 | 0.70 |
| 7 | 6.7 | 19.4 | 1.6 | 4.60 | 1.77 |
| 8 | 6.2 | 12.2 | 0.9 | 3.38 | 0.35 |
| 9 | 6.1 | 11.2 | 0.8 | 2.29 | 0.23 |
| 10 | 5.9 | 13.0 | 1.2 | 3.28 | 0.61 |
| 11 | 5.5 | 13.5 | 1.2 | 3.46 | 1.09 |
| 12 | 6.3 | 14.4 | 1.2 | 6.55 | 0.75 |
| 13 | 6.0 | 12.3 | 1.4 | 8.66 | 0.50 |
| 14 | 7.1 | 2.7 | 0.7 | 1.82 | 2.99 |
| 15 | 6.1 | 5.8 | 0.9 | 4.39 | 0.16 |
| 16 | 8.0 | 9.6 | 1.3 | 1.09 | 0.44 |
| 17 | 6.2 | 14.8 | 1.5 | 13.88 | 0.21 |
| 18 | 7.5 | 7.6 | 1.5 | 10.48 | 1.49 |
| 19 | 7.8 | 7.9 | 1.2 | 5.38 | 0.38 |
| 20 | 7.2 | 10.8 | 1.7 | 4.52 | 0.16 |
| 21 | 6.7 | 10.7 | 1.5 | 5.07 | 0.91 |
| 22 | 6.7 | 12.3 | 1.8 | 4.21 | 1.39 |
| 23 | 6.6 | 9.5 | 1.7 | 3.43 | 1.71 |
| 24 | 6.2 | 6.3 | 1.1 | 3.25 | 1.87 |
| 25 | 7.8 | 8.6 | 1.5 | 1.17 | 2.16 |

† All analytical data are reported as the mean of three replicate determinations.

‡ Soil to water ratio, 1:2.

§ Determined by wet combustion techniques, Nelson and Sommers (1996).

¶ Determined by permanganate-reduced iron modification of the semi-micro Kjeldahl procedure, Bremner (1996).

# Determined by steam distillation techniques, Mulvaney (1996).

achieved anaerobic conditions in <6 h (data not shown). Once anaerobic conditions were achieved, the soil was incubated for 14 d at 40°C.

After incubation, 50 mL of 4 $M$ KCl solution was added to the serum bottle, the bottle shaken for 1 h, and the soil solution mixture filtered through Whatman no. 42 filter paper (Whatman, Clifton, NJ). A 10-mL aliquot of the filtrate was steam distilled according to Mulvaney (1996) and the concentration of $NH_4$–N liberated from distillation was determined using acidimetric titration techniques.

## Hydrolyzable Amino Sugar-Nitrogen Procedure

Hydrolyzable amino sugar-N was determined according to the diffusion techniques of Mulvaney and Khan (2001). To prepare the soil hydrolysates, 5 g of oven-dried soil was combined with 20 mL of 6 $M$ HCl plus two drops of octyl alcohol and heated under reflux for 12 h at 110 to 120°C after treatment. The hydrolysis mixture was then filtered through Whatman no. 50 filter paper (Whatman, Clifton, NJ) under vacuum, after which the hydrolysates were stored at 5°C, until neutralized by addition of NaOH to obtain a pH of 6.4 to 6.8 (Stevenson, 1996).

To determine the concentration of hydrolyzable amino sugar-N, the amount of hydrolyzable $NH_4$–N was quantified first. Ten milliliters of the soil hydrolysate was placed in a modified Mason jar along with 0.05 g of MgO and heated on a hot plate at 48 to 50°C for 2 h, followed by the titrimetric determination of $NH_4$–N liberated. Next, the concentration of hydrolyzable ($NH_4$ + amino sugar)-N was quantified by placing 10 mL of soil hydrolysate in a modified Mason jar along with 2 mL of 10 $M$ NaOH and heated on a hot plate at 48 to 50°C for 5 h. Gaseous $NH_3$ was captured in $H_3BO_3$–indicator solution located in a Petri dish attached to the modified lid. The amount of $NH_4$–N captured in the indicator solution was quantified using acidimetric titration techniques.

The concentration of hydrolyzable amino sugar-N was then determined by the subtraction of the concentration of hydrolyzable $NH_4$–N from the concentration of hydrolyzable ($NH_4$ + amino sugar)-N.

## Analytical Methods for Predicting Hyrolyzable Amino Sugar-Nitrogen

The ISNT diffusion method was conducted according to Khan et al. (2001). One gram of oven-dried soil was treated with 10 mL of 2 $M$ NaOH in a modified 473 mL wide mouth Mason jar, and the sample was heated for 5 h at 48 to 50°C on a modified hot plate. Gaseous $NH_3$ was captured in $H_3BO_3$–indicator solution located in a Petri dish attached to the modified lid. The amount of $NH_4$–N captured in the indicator solution was quantified using acidimetric titration techniques.

Because most soil testing laboratories contain the equipment to perform steam distillation, the ISNT was conducted using steam distillation techniques in a similar fashion to the diffusion techniques of Khan et al. (2001). Instead of placing 1 g of soil in a modified Mason jar along with 10 mL of 2 $M$ NaOH, the soil was placed in a Kjeldahl flask along with 10 mL of 2 $M$ NaOH and steam distilled into $H_3BO_3$–indicator solution. After 35 mL of distillate was collected the amount of $NH_4$–N captured in the indicator solution was quantified using acidimetric titration techniques. Because of the concern of steam diluting the NaOH solution during distillation, increasing concentrations of 5 and 10 $M$ NaOH solutions were also analyzed.

## Recovery of Glucosamine from soil using the Illinois Soil Nitrogen Test and 10 $M$ NaOH Steam Distillation

For use in the recovery test, reagent-grade glucosamine was obtained from Sigma (Milwaukee, WI) and an aqueous solution was prepared containing 200 mg N L$^{-1}$ and stored in a refrigerator for use within 24 h after preparation. A subsample of the glucosamine solution was digested using semimicro-Kjeldahl/steam distillation techniques (Bremner, 1996) to determine the exact N concentration.

Four soils (2, 4. 15, and 18) were chosen based on soil texture, ISNT value and geographic region for use in the recovery test. One gram soil samples were treated with 0 or 200 μg N of the glucosamine solution and in <30 min subject to analysis by the ISNT and 10 $M$ NaOH steam distillation methods with each treatment replicated four times. Amino sugar-N recovery was determined by the difference method between the 0 and 200 μg N rates for each soil.

## Statistical Analysis

For all analytical procedures the mean response was calculated from three replicates and regressed against the mean response of the analytical procedure of interest. Simple regression techniques were used to determine linear relationships between methods. All statistical analyses were performed using SAS Version 8.2 (SAS Institute, Inc., Cary, NC).

## RESULTS AND DISCUSSION

The need for a soil test that accurately predicts N mineralization has increased due to fertilizer costs and environmental concerns. The most consistent and reliable methods have been biological indices, that is, anaerobic or aerobic incubation of the soil (Keeney, 1982; Bundy and Meisinger, 1994; Wilson et al., 1994). These methods, however, do not lend themselves to routine use in a soil-test laboratory because of the time needed to incubate the soil. Nevertheless, the laboratory incubation procedures are good standards to evaluate new analytical methods for estimating soil N mineralization potential. Recently, Mulvaney et al. (2001) reported that high concentrations of hydrolyzable amino sugar-N in the soil reduced the need for N fertilization. If the hydrolyzable amino sugar-N fraction is the N fraction that controls labile-N availability in the soil, then it would seem rational that the amino sugar-N fraction would correlate well with $NH_4$–N mineralized during a 14 d anaerobic incubation. Additionally, any method that accurately predicted hydrolyzable amino sugar-N should be an accurate predictor of N mineralization.

## Hydrolyzable Amino Sugar-Nitrogen as an Index of Nitrogen Availability

In this study, hydrolyzable amino sugar-N was compared to $NH_4$–N mineralized during anaerobic incubation, which has been reported to be a reliable predictor of N mineralization (Keeney, 1982; Bundy and Meisinger, 1994). Hydrolyzable amino sugar-N concentrations in the 25 soils ranged from 81.4 to 423.7 mg kg$^{-1}$, which were greater than the 9.1 to 164.5 mg $NH_4$–N kg$^{-1}$ mineralized during the 14 d anaerobic incubation (Table 3). When hydrolyzable amino sugar-N values were regressed vs. the $NH_4$–N mineralized after the 14 d of anaerobic incubation (Fig. 1A), a significant linear relationship was observed. Even though the relationship was significant, hydrolyzable amino sugar-N explained only 38% of the varia-

tion in $NH_4$–N mineralized after 14 d. With this low coefficient of determination, it may be assumed that other fractions of N, perhaps along with the hydrolyzable amino sugar-N fraction, are contributing to N mineralization. Because the hydrolyzable $NH_4$–N has to be determined to quantify the concentration of hydrolyzable amino sugar-N (Mulvaney et al., 2001), the hydrolyzable $NH_4$–N concentrations, ranging from 89.1 to 377.8 mg $kg^{-1}$, along with hydrolyzable ($NH_4$ + amino sugar)-N concentrations, ranging from 177.5 to 704.7 mg $kg^{-1}$ (Table 3), were regressed against the $NH_4$–N mineralized during anaerobic incubation. Hydrolyzable $NH_4$–N alone only accounted for 50% of the variability (Fig. 1B), however, when combined with hydrolyzable amino sugar-N (Fig. 1C) the coefficient of determination ($R^2$ = 0.61) was greater than that of both the hydrolyzable $NH_4$–N and the hydrolyzable amino sugar-N linear models. These results may imply that amino sugar-N along with other more labile organic N pools are contributing to the mineralizable N potential for soils.

Table 3. Means and standard deviations for methods used.†

| Soil no. | Anaerobic incubation | Hydrolyzable (AS‡ + NH4)-N | Hydrolyzable AS-N | Hydrolyzable NH4–N | ISNT§ | SD ¶ 10 M NaOH |
|---|---|---|---|---|---|---|
| | | | —mg $kg^{-1}$— | | | |
| 1 | 27.4 ± 9.1 | 201.2 ± 13.4 | 81.4 ± 13.4 | 119.8 ± 6.9 | 71.5 ± 4.9 | 69.5 ± 8.8 |
| 2 | 9.1 ± 4.0 | 177.5 ± 13.9 | 86.6 ± 13.9 | 90.9 ± 9.5 | 63.1 ± 11.6 | 64.1 ± 3.9 |
| 3 | 71.8 ± 10.3 | 437.0 ± 24.3 | 183.6 ± 24.3 | 253.5 ± 5.4 | 185.9 ± 9.0 | 183.4 ± 7.0 |
| 4 | 130.2 ± 8.8 | 704.7 ± 3.0 | 423.7 ± 3.0 | 281.0 ± 11.2 | 297.1 ± 3.6 | 301.0 ± 6.8 |
| 5 | 85.7 ± 9.3 | 632.7 ± 20.5 | 257.1 ± 20.5 | 377.8 ± 6.7 | 266.9 ± 19.5 | 219.5 ± 10.6 |
| 6 | 76.6 ± 3.6 | 521.7 ± 23.4 | 203.4 ± 23.4 | 318.4 ± 21.9 | 238.0 ± 13.9 | 221.9 ± 3.5 |
| 7 | 100.5 ± 11.7 | 544.3 ± 11.0 | 227.5 ± 11.0 | 316.7 ± 12.2 | 249.9 ± 11.5 | 226.9 ± 4.0 |
| 8 | 92.8 ± 3.0 | 259.5 ± 15.0 | 109.3 ± 15.0 | 150.2 ± 19.5 | 149.3 ± 9.0 | 132.2 ± 5.2 |
| 9 | 74.4 ± 7.3 | 301.3 ± 26.0 | 113.3 ± 26.0 | 188.0 ± 24.0 | 126.7 ± 5.8 | 121.3 ± 4.8 |
| 10 | 109.7 ± 9.6 | 337.9 ± 23.5 | 133.7 ± 23.5 | 204.2 ± 1.6 | 171.3 ± 15.2 | 168.4 ± 7.6 |
| 11 | 98.2 ± 5.3 | 337.0 ± 31.7 | 170.4 ± 31.7 | 166.6 ± 11.3 | 153.2 ± 7.6 | 145.9 ± 4.2 |
| 12 | 83.8 ± 5.8 | 407.5 ± 9.6 | 173.6 ± 9.6 | 233.9 ± 24.9 | 186.2 ± 12.2 | 170.7 ± 10.3 |
| 13 | 115.0 ± 9.7 | 431.6 ± 5.4 | 227.4 ± 5.4 | 204.2 ± 16.3 | 217.1 ± 4.4 | 194.9 ± 3.7 |
| 14 | 27.8 ± 0.2 | 278.8 ± 23.8 | 189.7 ± 23.8 | 89.1 ± 4.7 | 102.8 ± 2.4 | 104.8 ± 4.2 |
| 15 | 50.4 ± 9.6 | 340.5 ± 2.7 | 190.3 ± 2.7 | 150.2 ± 12.1 | 112.5 ± 11.9 | 124.0 ± 8.4 |
| 16 | 64.3 ± 2.5 | 407.9 ± 7.9 | 138.5 ± 7.9 | 265.2 ± 12.3 | 167.7 ± 10.8 | 116.6 ± 2.0 |
| 17 | 146.2 ± 2.5 | 462.9 ± 21.7 | 175.0 ± 21.7 | 287.9 ± 13.6 | 230.9 ± 5.2 | 221.9 ± 3.2 |
| 18 | 110.6 ± 5.4 | 481.7 ± 22.5 | 283.8 ± 22.5 | 197.9 ± 15.6 | 187.5 ± 16.7 | 190.8 ± 4.7 |
| 19 | 97.1 ± 3.7 | 395.9 ± 30.5 | 243.8 ± 30.5 | 152.1 ± 8.6 | 153.5 ± 9.1 | 146.4 ± 4.4 |
| 20 | 116.2 ± 9.0 | 590.7 ± 30.9 | 332.5 ± 30.9 | 258.2 ± 13.9 | 239.1 ± 16.4 | 212.3 ± 7.6 |
| 21 | 133.5 ± 2.6 | 528.5 ± 18.4 | 201.6 ± 18.4 | 329.8 ± 7.7 | 176.8 ± 11.2 | 172.1 ± 5.3 |
| 22 | 164.5 ± 8.8 | 593.2 ± 18.7 | 251.4 ± 18.7 | 345.4 ± 9.6 | 248.8 ± 15.8 | 199.8 ± 4.0 |
| 23 | 148.4 ± 4.5 | 575.5 ± 20.8 | 331.7 ± 20.8 | 243.8 ± 27.0 | 224.2 ± 13.0 | 216.4 ± 7.6 |
| 24 | 91.4 ± 7.6 | 428.9 ± 16.7 | 227.4 ± 16.7 | 201.5 ± 34.4 | 140.3 ± 10.0 | 145.9 ± 9.8 |
| 25 | 119.3 ± 6.5 | 535.1 ± 10.2 | 208.2 ± 10.2 | 323.2 ± 19.3 | 189.4 ± 13.7 | 146.7 ± 7.2 |

† All analytical data are reported as the mean of three replicate determinations ± one standard deviation.
‡ AS, amino sugar.
§ ISNT, Illinois soil nitrogen test.
¶ SD, steam distillation.

Our results differ from those reported by Mulvaney et al. (2001), which found no significant correlation between corn check-plot yield or N-fertilizer response and hydrolyzable $NH_4$–N concentrations on Illinois soils. Mulvaney et al. (2001) made no attempt to correlate hydrolyzable ($NH_4$ + amino sugar)-N concentrations with corn check-plot yield or N-fertilizer response. Their best correlation among the soil chemical properties evaluated with check-plot yield and N-fertilizer response was hydrolyzable amino sugar-N.

## Comparison of Analytical Techniques to Predict Hydrolyzable Amino Sugar-Nitrogen

Due to the popularity of steam distillation, the ISNT diffusion method was conducted using steam distillation techniques in a similar fashion to the diffusion techniques of Khan et al. (2001). Because of the concern of the steam diluting the 2 $M$ NaOH solution during distillation, increasing concentrations of 5 and 10 $M$ NaOH were also evaluated. When the ISNT and steam distillation values were regressed vs. hydrolyzable amino sugar-N concentrations significant linear relationships were observed (Table 4). Even though the coefficients of determination for the aforementioned methods with the hydrolyzable amino sugar-N method were high ($R^2$ = 0.45 to 0.63), they were not as high as the coefficient of determination measured by Khan et al. (2001) with the ISNT method ($R^2$ = 0.82). The higher coefficient of determination obtained

by Khan et al. (2001) may be due to the small number of soils studied, in which all samples were from the same geographic location and no clayey textured soils were analyzed.

As previously stated, the concentration of hydrolyzable $NH_4$–N and hydrolyzable ($NH_4$ + amino sugar)-N correlated better with N mineralized during the 14 d anaerobic incubation experiment than hydrolyzable amino sugar-N alone (Fig. 1). So when the four analytical methods were compared to the concentration of hydrolyzable $NH_4$–N (Table 4) coefficients of determination improved, however, when compared to hydrolyzable ($NH_4$ + amino sugar)-N the coefficient of determination improved further (Fig. 2). All four analytical methods displayed highly significant relationships ($P < 0.0001$). The ISNT method predicted hydrolyzable ($NH_4$ + amino sugar)-N best overall, but the 2, 5, and 10 NaOH steam distillation procedures also had acceptably high correlations (Fig. 2). These results were similar to those observed by Barker et al. (2006), in which they observed hydrolyzable ($NH_4$ + amino sugar)-N had the highest correlation with the ISNT ($R^2$ = 0.87), followed by hydrolyzable $NH_4$–N ($R^2$ = 0.84) and hydrolyzable amino sugar-N ($R^2$ = 0.33), respectively.

## Comparison of Analytical Techniques to Predict Anaerobic Incubation

We observed that the four evaluated analytical methods correlated with the soil organic N fraction that had significant relationships with potentially mineralizable soil N. So, it would
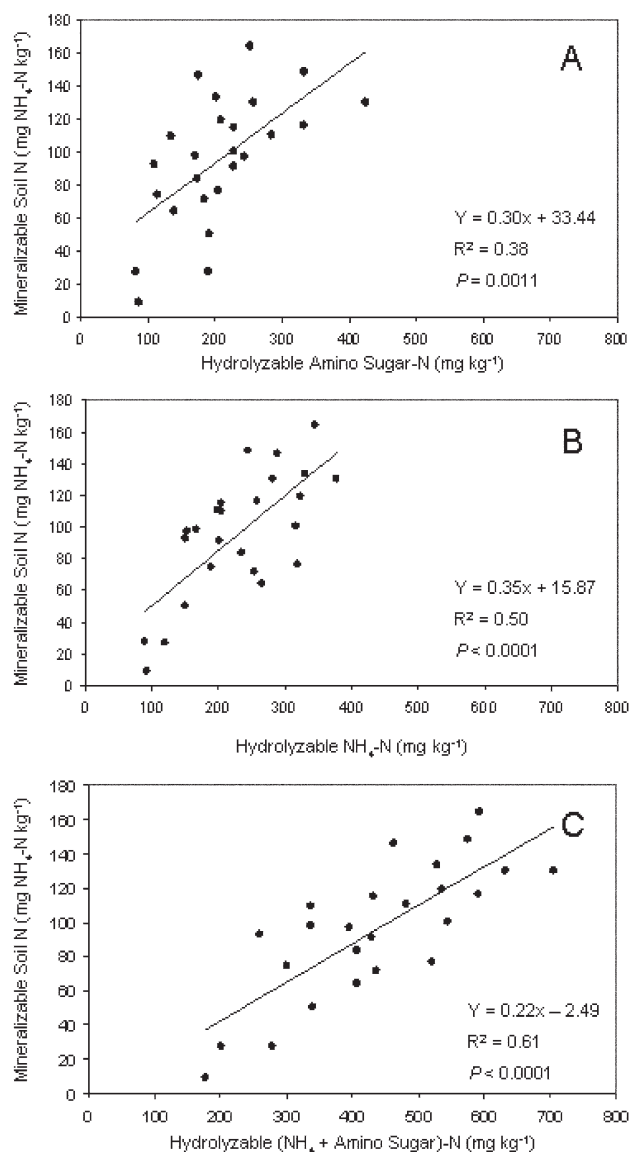
**Fig. 1. Linear regression models for hyrdrolyzable $NH_4$–N, hydrolyzable amino sugar-N, and hydrolyzable ($NH_4$ + amino sugar)-N vs. $NH_4$–N mineralized during a 14 d anaerobic incubation.**

seem reasonable that these more time efficient analytical methods would have strong relationships with $NH_4$–N mineralized

**Table 4. Linear models describing the relationship between hydrolyzable $NH_4$–N and amino sugar-N compared to four analytical methods.**

| Model | *P* value | $R^2$ | Slope | Intercept |
|---|---|---|---|---|
| | Hydrolyzable amino sugar-N | | | |
| ISNT† | <0.0001 | 0.52 | 0.93 | 35.35 |
| SD‡ 2 *M* NaOH | 0.0003 | 0.45 | 1.52 | 39.98 |
| SD 5 *M* NaOH | <0.0001 | 0.55 | 1.32 | 25.50 |
| SD 10 *M* NaOH | <0.0001 | 0.63 | 1.17 | 8.66 |
| | Hydrolyzable $NH_4$–N | | | |
| ISNT | <0.0001 | 0.70 | 1.09 | 30.48 |
| SD 2 *M* NaOH | <0.0001 | 0.62 | 1.81 | 31.75 |
| SD 5 *M* NaOH | <0.0001 | 0.57 | 1.35 | 45.02 |
| SD 10 *M* NaOH | <0.0001 | 0.50 | 1.05 | 51.95 |

† ISNT, Illinois soil nitrogen test.

‡ SD, steam distillation.

during anaerobic incubation. When the soil $NH_4$–N liberated with the ISNT and the 2, 5, and 10 *M* NaOH steam distillation methods were regressed against the $NH_4$–N mineralized after the 14 d anaerobic incubation (Fig. 3), significant linear relationships were observed ($P < 0.0001$). Coefficients of determination were all very similar with values of approximately 0.60. These correlation values were not as high as those observed by Khan et al. (2001), but could potentially be higher because as stated earlier the soils used in our study had different physical and chemical properties and came from a large geographic area. Bushong et al. (2007) also noted that when 50 soils with different chemical properties and geographic origins were reduced to 16 silt loam soils from the eastern one-half of Arkansas, coefficients of determinations for statistical models for comparing the ISNT and potentially mineralizable soil N increased from 0.45 to 0.71. The ISNT and 10 *M* NaOH steam distillation technique had smaller slope values than the 2 and 5 *M* NaOH steam distillation techniques. This decreased slope indicates a wider operating range for the ISNT and the 10 *M* NaOH steam distillation technique, thus potentially making it easier to detect differences in mineralizable soil N. Sharifi et al. (2007) also observed significant relationships between a 24 wk aerobic incubation and the ISNT ($R^2 = 0.51$) and their direct steam distillation procedure ($R^2 = 0.61$) using soils from Canada and the northeast United States.

## Comparison of the Illinois Soil Nitrogen Test and Direct Steam Distillation Techniques

The ISNT method has two limitations for use in large-scale soil testing laboratories. The ISNT method requires a large amount of space to place the diffusion chambers and requires a 5 h heating time to complete the diffusion. Thus, we investigated the use of steam distillation as a viable option to the ISNT diffusion technique. When the 2, 5, and 10 *M* NaOH distillation techniques were regressed against the ISNT (Fig. 4), highly significant relationships were observed for all concentrations of NaOH steam distilled ($P < 0.0001$). Direct steam distillations for all three strengths of NaOH displayed coefficients of determination of approximately 0.90 when compared to the ISNT diffusion method. These results were similar to those observed by Sharifi et al. (2007), who observed a strong correlation between the ISNT and their direct steam distillation method ($R^2 = 0.92$). The parameters that set the models apart in this study were the slope and intercept values. As the concentration of NaOH increased the slopes decreased. The 10 *M* NaOH distillation displayed a slope of 1.08 and an intercept of 1.10 when regressed with the ISNT. With a slope close to one and an intercept near zero, the 10 *M* NaOH method produced values almost identical to the ISNT values. Thus, soil testing laboratories have an option of using the ISNT diffusion method or the 10 *M* NaOH steam distillation procedure to quantify amino sugar-N as well as hydrolyzable $NH_4$–N.

## Comparison of the Illinois Soil Nitrogen Test and Steam Distillation for Glucosamine-Nitrogen Recovery from Soil

Percent recovery of glucosamine-N from the soil was significantly affected by the soil type and method as shown in the ANOVA *P* values presented in Table 5. There was not a significant soil by method interaction as the ISNT consistently
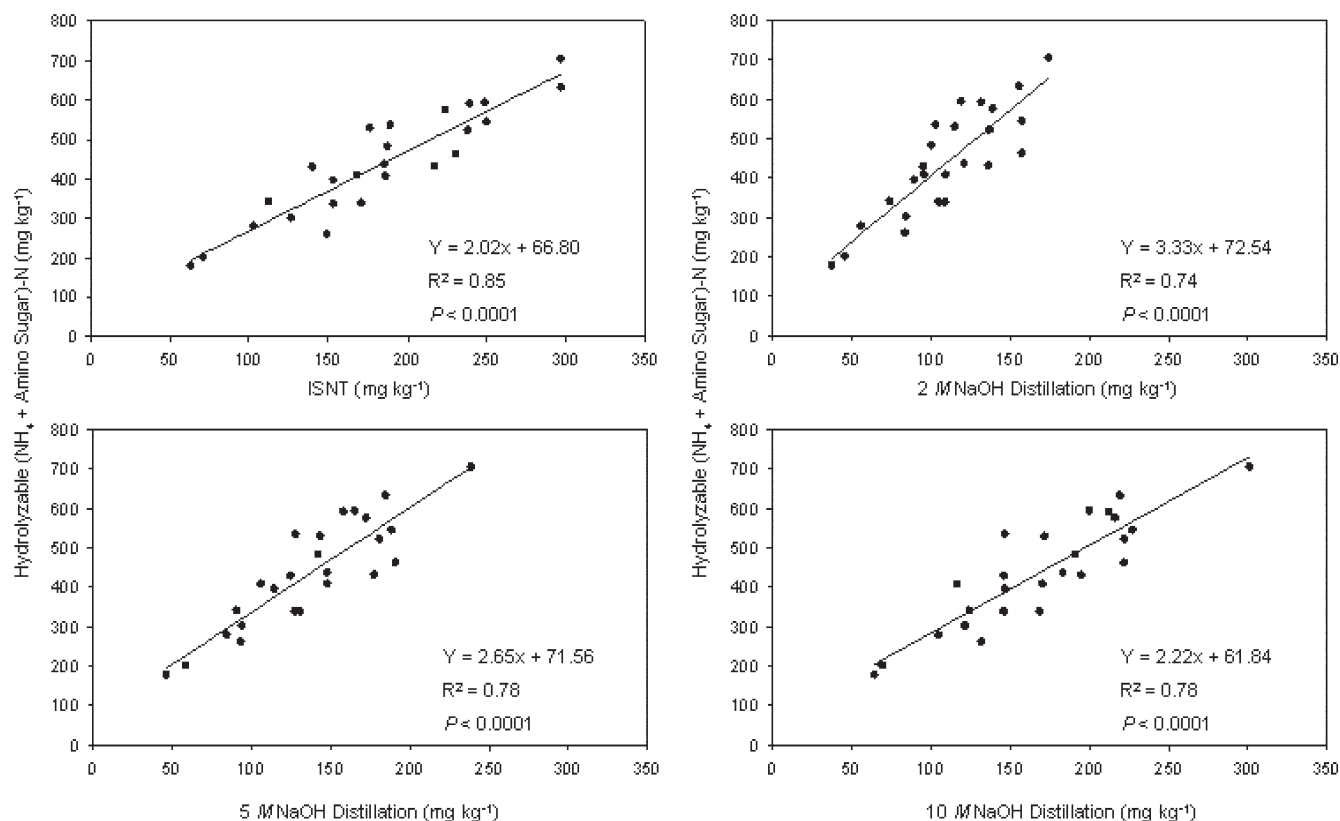
**Fig. 2. Linear regression models for the Illinois soil nitrogen test (ISNT), and steam distillation techniques using 2, 5, and 10 *M* NaOH vs. hydrolyzable (NH₄ + amino sugar)-N.**
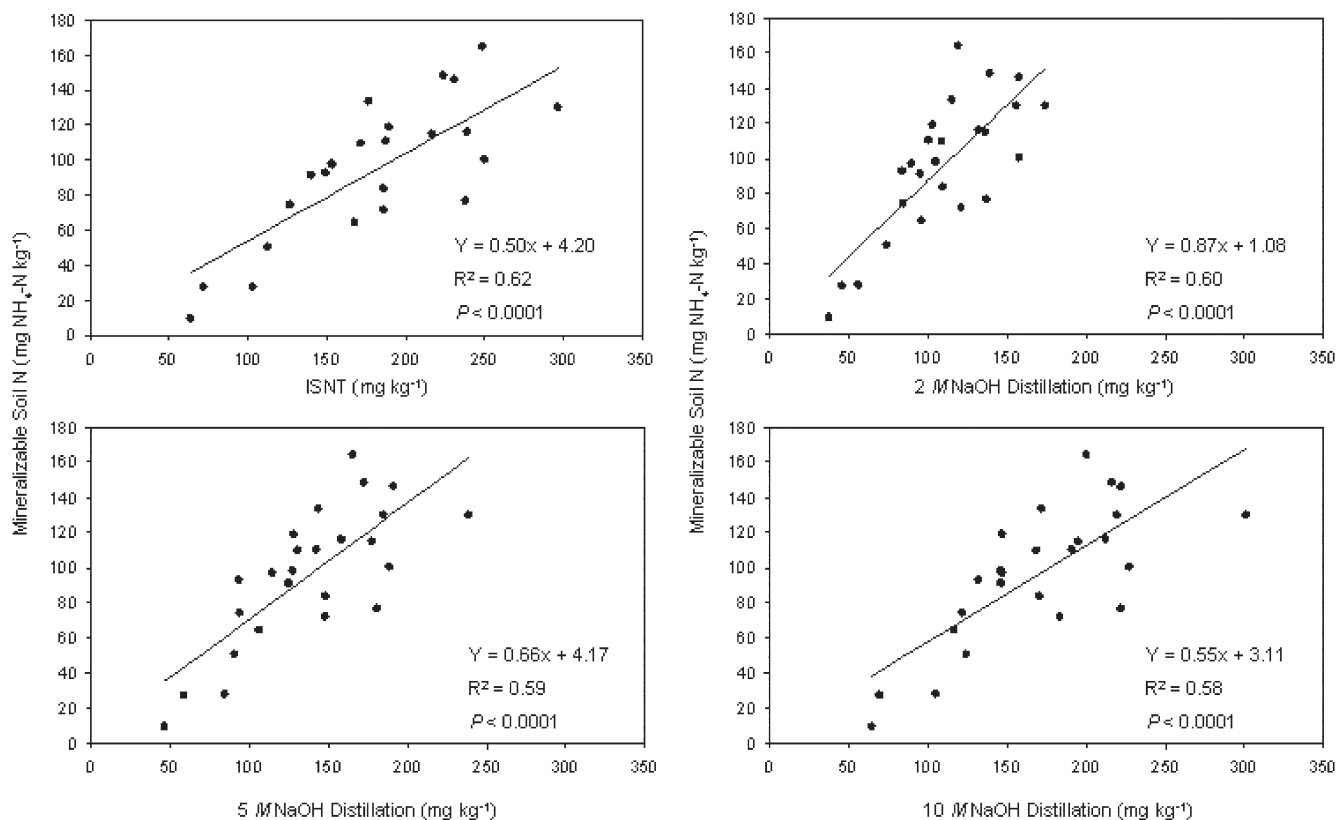


**Fig. 3. Linear regression models for the Illinois soil nitrogen test (ISNT), and steam distillation techniques using 2, 5, and 10 *M* NaOH vs. NH₄–N mineralized during 14 d anaerobic incubation.**
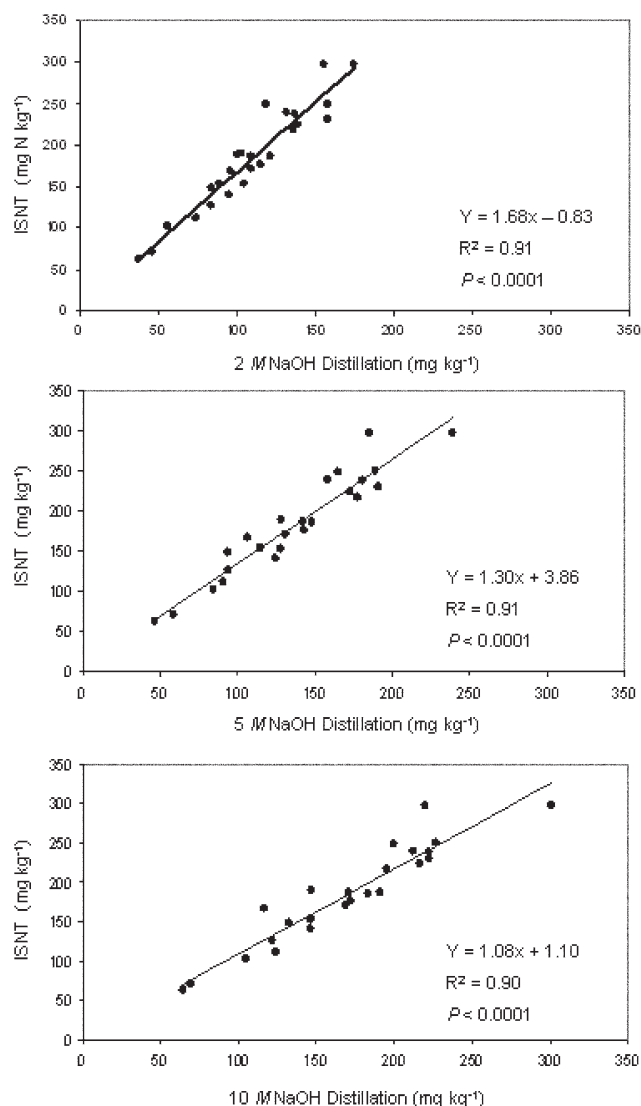
**Fig. 4. Linear regression models for steam distillation techniques using 2, 5, and 10 _M_ NaOH vs. the Illinois soil nitrogen test (ISNT).**

**Table 5. Analysis of variance _P_ values for percent spike recovery as affected by soil, method, and their interaction.**

| Source | Degrees of freedom | Spike recovery, % |
|---|---|---|
| | | –_P_ value– |
| Soil | 3 | <0.0001 |
| Method | 1 | <0.0001 |
| Soil × method | 3 | 0.229 |

**Table 6. Comparison of the Illinois soil nitrogen test (ISNT) and 10 _M_ NaOH steam distillation for percent recovery of 200 µg N glucosamine-N spike in selected soils.**

| | ISNT | | 10 M NaOH steam distillation | |
|---|---|---|---|---|
| Soil | Mean | STD | Mean | STD |
| | –% recovery of glucosamine spike– | | | |
| 2 | 90.56 | 2.16 | 85.89 | 0.98 |
| 4 | 94.91 | 1.88 | 92.34 | 1.89 |
| 15 | 92.08 | 2.38 | 86.46 | 0.85 |
| 18 | 93.47 | 0.72 | 90.35 | 0.80 |

LSD(0.05) within soil 1.95
LSD(0.05) among soil 2.74

recovered a higher percentage of glucosamine-N than did the 10 _M_ NaOH steam distillation independent of soil type. There is an observed difference in glucosamine-N recovery among soils (Table 6) with an $LSD_{(0.05)}$ = 2.74%. Soil texture appears to interfere with glucosamine-N recovery as the coarse textured soil resulted in the lowest recovery of glucosamine-N, whereas the finer textured soil resulted in a significantly higher recovery. The two medium textured soils were not different than either the clay or sandy textured soil. Although significant differences in glucosamine-N recovery were observed among soils, there was a high recovery ( >85%) for both methods across all soils. The ISNT recovers more glucosamine-N than steam distillation, but the standard deviations for a given soil are lower for steam distillation. Close observation shows a much wider range in recoveries based on soil for steam distillation, but with a higher amount of precision within a particular soil (Table 6). Both methods recover a high level of glucosamine-N from the soil strengthening their ability to estimate amino sugar-N or potentially mineralizable-N from the soil with an adequate degree of accuracy.

## CONCLUSIONS

In conclusion, if the $NH_4$–N mineralized after 14 d of anaerobic incubation is a reliable indicator of N mineralization, then hydrolyzable amino sugar-N is also an accurate predictor of N mineralization as previously stated by Mulvaney et al. (2001). However, when the hydrolyzable amino sugar-N concentrations were combined with the hydrolyzable $NH_4$–N concentrations the ability to predict N mineralization improved, suggesting that other labile soil N forms along with amino sugar-N are potentially becoming plant available. Rapid analytical procedures like the ISNT diffusion method and the 2, 5, and 10 _M_ NaOH steam distillation techniques accurately predicted hydrolyzable amino sugar-N as well as hydrolyzable ($NH_4$ + amino sugar)-N. These methods also accurately predicted $NH_4$–N mineralized during anaerobic incubation. It could be assumed that this predictability may increase when soils are analyzed based on texture and geographic origin. Recovery and quantification of amino sugar-N from the soil was high for both methods. Although the ISNT recovered slightly more amino sugar-N, steam distillation has a higher level of precision for a given soil. Results show that both methods can be used to estimate amino sugar-N in soil and can be used to predict potentially mineralizable-N. Thus, in soil-testing facilities where the ISNT is already implemented as a procedure, the much quicker and equally reliable 10 _M_ NaOH distillation technique may be used to achieve near identical test values.

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